*Kwik*Western Buffer

(Cat.# R2002)

A new buffer system for Western Blotting:

1. Blocking with skim milk or BSA is not needed if using this buffer. Dilute the primary or secondary antibody in this buffer, and use it directly.

2. The antibodies can be re-used directly. No preservative is needed. Store the recycled antibody solution in either 4C or -20C.

This buffer produces cleaner blots than regular TBST does.

IMPORTANT: rinse the blot with dI-water before applying ECL substrate solution. This buffer contains a compound that slightly inhibits HRP.

(Protocol on the next page)

<u>Preparation</u>: Add component A and B in 10L water. Shake 10-15 minutes to dissolve all components. Store at room temperature.

Protocol:

1. Dilute the primary antibody in the R2002 at the desired concentration.
2. Submerge the membrane (after the transfer step) in the solution. Blocking the membrane with milk or BSA is not necessary.
3. Incubate the membrane at 4C or 25C for a desired duration.
4. Remove the membrane, and store the antibody at 4C or -20C for next time use.
5. Submerge the membrane in sufficient R2002, and put the container on a shaker. Note the membrane MUST move freely while shaking.
6. Wash the membrane for **<u>30 minutes</u>** at room temperature, and decant the buffer.
7. Repeat steps 5 and 6 for **<u>three (3)</u>** more times.
8. Rinse the membrane with dI water for 15 sec.
9. Apply ECL substrate to the membrane for imaging.

   (The blot background should be very low. If the background is high, put the membrane back in the buffer and continue washing for another 30-60 minutes. If the additional background can't bring down the background, the cause of the background is very likely from the primary antibody.)